

XIV. "Researches illustrative of the Physico-Chemical Theory of Fermentation, and of the conditions favouring Archebiosis in previously Boiled Fluids."—No. I. By H. CHARLTON BASTIAN, M.A., M.D., F.R.S., Professor of Pathological Anatomy in University College, London, and Physician to University College Hospital. Received May 25, 1876.

(Abstract.)

The author first calls attention to the fact that no previous investigator has professed to have seen well-marked fermentation set up in urine that had been boiled for a few minutes, if it has thereafter been guarded from contamination. The previous invariable barrenness of this fluid after boiling has been ascribed by germ-theorists to the fact that any organisms or germs of organisms which it may have contained were killed by raising it to the temperature of 212° F. (100° C.).

In executing some of the experiments with urine described in this communication, two chemical agents have been brought into operation under novel conditions, and an ordinary physical influence has been employed to an entirely new extent. In several respects, therefore, these new experiments differ much, as regards the conditions made use of, from those hitherto devised for throwing light upon the much-vexed questions as to the possible origin of fermentations independently of living organisms or germs, and as to the present occurrence or non-occurrence of Archebiosis.

The chemical agents employed under new conditions in these experiments were *liquor potassæ* and *oxygen*—both of them being well known as stimulants, if not as promoters, of many fermentative processes.

It has been recognized by several investigators of late years that neutral or slightly alkaline organic fluids are rather more prone to undergo fermentation than slightly acid fluids. This fact may be easily demonstrated. As the author pointed out in 1870, if two portions of an acid infusion are exposed side by side at a temperature of 77° F. (25° C.) fermentation may be made to appear earlier and to make more rapid progress in either of them by the simple addition of a few drops of liquor potassæ; on the other hand, if a neutral infusion be taken and similarly divided into two portions placed under the same conditions, fermentation may be retarded or rendered slower in either of them at will by the simple addition to it of a few drops of acetic or some other acid.

A neutral or faintly alkaline organic solution can in this way be demonstrated to possess a higher degree of fermentability than an otherwise similar acid organic solution. It seems, therefore, obvious that the changes capable of taking place in *boiled* acid and neutral solutions respectively should also vary considerably. Numerous experiments by

different observers have demonstrated the correctness of this inference. Boiled acid infusions guarded from contamination mostly remain pure and barren if kept at temperatures below 77° F. (25° C.), though other infusions similarly treated and similar in themselves, except that they have been rendered neutral by an alkali, will oftentimes become corrupt and swarm with organisms. The latter result follows still more frequently with neutral infusions when they are exposed to a higher generating temperature in the warm-air chamber; and under this stronger stimulus a small number of boiled acid fluids will also ferment.

On the other hand, the influence of oxygen in promoting fermentation has been fully appreciated since the early part of the present century. Formerly an influence was assigned to it as an initiator of fermentation as all-important as some chemists assign to living germs at the present day. But this was a very exaggerated view. In some fluids, as the author has shown, fermentation may be initiated just as freely, or even rather more so, in closed vessels from which the air has been expelled by boiling, as in others in which atmospheric air, and consequently oxygen, is present. The explanation of this fact is probably to be found in the supposition that, in starting the fermentation of these fluids, diminution of pressure may be of as much, or even of more, importance than contact with free oxygen. In respect to other organic fluids, however, the influence of oxygen seems decidedly more potent as a co-initiator of fermentation than that diminution of pressure which is brought about by hermetically sealing the vessel before the fluid within has ceased to boil. Urine will be found to be an example of this latter class of fluids.

The physical influence which has been employed in unusual intensity in the present researches is *heat*.

Previous experimenters have never designedly had recourse to a generating or developing temperature above 100° F. (38° C.). The heat employed has frequently been below 77° F. (25° C.), though a temperature between this and 95° F. (35° C.) has been regarded both by chemists and biologists as most favourable to the occurrence and progress of fermentative changes generally.

Early in the month of August 1875 the author ascertained the fact that some boiled fluids which remained barren when kept at a temperature of 77°–86° F. (25°–30° C.) would rapidly become turbid and swarm with organisms if maintained at a temperature of 115° F. (46° C.). More recently he has discovered the surprising fact that a generating temperature as high as 122° F. (50° C.) may be had recourse to with advantage in dealing with some fermentable solutions. Fluids which would otherwise have remained barren and free from all signs of fermentation have under the influence of this high temperature rapidly become turbid and corrupt. This discovery is regarded as of great importance in reference to the questions now under discussion, and it is one which was quite unexpected. The author had previously shared in

the generally received opinion that temperatures above 100° F. (38° C.) were likely to impede rather than promote fermentation.

In maintaining the experimental fluids at the high temperature above named, the vessels containing them were placed in the hot-air chamber of an incubator such as physiologists employ, to which one of the very ingenious gas-regulators of Mr. F. J. Page had been fitted (see *Journal of the Chemical Society*, January, 1876). In this way the fluids may be kept at a known and practically constant temperature for an indefinite time.

Liquor Potassæ as a promoter of Fermentation in Boiled Urine.

In the autumn of 1875 the author instituted some experiments to ascertain whether the fermentability of boiled urine, like that of many other fluids, could be increased by previously mixing with it a quantity of liquor potassæ sufficient for its neutralization.

The experiments answered this question in the affirmative. It was found that urine to which the above-named amount of liquor potassæ had been added would constantly ferment and swarm with organisms within a few days after it had been boiled, though some of the same stock of urine in the acid state (that is, without the addition of any alkali) would when similarly treated in other respects remain barren. The fact of the production of an increased fermentability in boiled urine by previous neutralization was thus established.

Further experiments were then instituted to throw light upon the cause of such increased fermentability. It was desirable to ascertain whether (1) it was due to survival of germs in the boiled neutralized fluid, or (2) to the chemical influence of potash in initiating or helping to initiate the molecular changes leading to fermentation in a fluid devoid of germs or other living matter.

The mode of testing the relative validity of these rival interpretations seemed easy. It was only necessary to ascertain what the effect would be of adding boiled liquor potassæ, in proper quantity, after the acid urine had been rendered barren by boiling it, instead of adding it previous to the process of ebullition. If fermentation occurred in the fluid thus neutralized without extraneous contamination, the first interpretation would obviously be negatived.

This crucial experiment was at first tried with flasks plugged with cotton-wool, the plug in each of them being penetrated by a closed glass tube containing the measured amount of liquor potassæ. The tubès having been drawn out to a capillary portion at the lower end, and bent at an obtuse angle, they could be easily broken by slight downward pressure against the bottom of the flask whenever it was desired to mix the liquor potassæ with the boiled urine. This apparatus was very similar to that first made use of by Dr. William Roberts in some experiments with hay-infusion (*Phil. Trans.* vol. clxiv. p. 474), in which he obtained opposite results from those now about to be recorded with urine. The

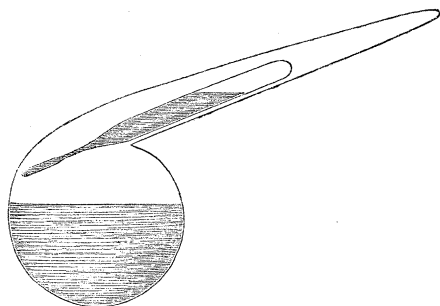
latter fluid, however, is for several reasons more suitable than hay-infusion for trying such experiments.

Several trials made with urine in this apparatus showed that its fermentability was just as much increased by adding boiled liquor potassæ after the urine had been boiled in the acid state, as by adding the alkali previous to the process of ebullition. Such a result was therefore quite opposed to the first interpretation as to the cause of the increased fermentability of neutralized urine.

The definite overthrow or establishment of this interpretation was so important that it seemed desirable to try such experiments again by some more rigid and certain method. The author, therefore, devised a new mode of experimentation in which sealed retorts replaced the flasks plugged with cotton-wool, and in which the contents of the enclosed liquor-potassæ tubes could be more effectually heated.

It was first of all ascertained that accurately neutralized urine boiled in a retort and sealed whilst boiling would ferment in a day or two if kept at a temperature of 122° F. *

This fact having been established, other retorts were charged with a measured amount of urine, and also with a small glass tube containing liquor potassæ in quantity almost sufficient to neutralize the urine employed †. The glass tubes containing the liquor potassæ had been drawn out at one end, sealed and then immersed in boiling water for different periods before introducing them into the retorts. After each



retort had been charged with urine and a liquor-potassæ tube, its neck was drawn out to a capillary point, the urine was boiled, and the retort was hermetically sealed before ebullition had ceased. Thus closed, the

* Though the boiled urine will ferment in retorts from which the air has been expelled by boiling, it will undergo this change more quickly if it is in the presence of purified or sterilized air. In the experiments now about to be described, however, it was much more convenient to use airless retorts.

† As a slight excess in the amount of liquor potassæ has been proved to have a most restrictive influence when dealing with urine, it was found safer in these experiments not to provide liquor potassæ sufficient for full neutralization. Many details on this subject are given in the memoir itself.

vessel was at once immersed with its neck downwards in a can of boiling water for from four to fifteen minutes, so as to expose it and its contents for an additional period to a temperature of 212° F. (100° C.).

The urine was thus boiled in its unaltered acid state and sterilized. After the retorts had cooled the liquor potassæ was liberated from its tube in all but one of the batch, which was kept as a control experiment. The liberation was easily effected. It was only necessary to give the retort a sudden shake so as to drive the capillary neck of the enclosed tube against its side. The tube was thus broken and immediately (owing to the comparative vacuum within the retort) the liquor potassæ was sucked out and mixed with the fluid which it was destined to neutralize.

The result of these experiments was similar to those executed with the plugged flasks and liquor-potassæ tubes. The boiled caustic potash added afterwards within the sealed retorts caused the previously barren fluids to ferment and swarm with *Bacteria*. The fluid in the control experiment remained pure, though after several days, or longer, it also could be made to ferment by breaking the liquor-potassæ tube and replacing the retort in the warm chamber.

Effects of liberating Oxygen by Electrolysis within the closed Retorts.—A few other experiments were made with retorts to which platinum electrodes had been fitted. These contained, as before, measured amounts of urine, together with liquor-potassæ tubes. All the preliminary stages were similar to those of the experiments above recorded; but just before breaking the liquor-potassæ tubes in these further experiments, oxygen and hydrogen were liberated from the boiled urine by electrolysis.

The result in the few experiments made was very remarkable. Under the combined influence of liquor potassæ, oxygen, and the high temperature of 122° F. (50° C.), the sterilized urine fermented and swarmed with *Bacteria* within the closed retorts in from 7–12 hours—that is, in a much shorter time than would suffice for the occurrence of similar changes in unboiled urine freely exposed to the air.

Behaviour of some specimens of unaltered Acid Urine under the influence of the High Generating Temperature of 122° F. (50° C.).

In the course of the previous experiments it was found that occasionally a specimen of boiled urine would ferment at a temperature of 122° F. without the addition of liquor potassæ. This was afterwards ascertained to occur invariably (with the urine experimented upon) when the acidity of the fluid was not higher than would be represented by six minims of liquor potassæ to the ounce (or about 1½ per cent.). Urines slightly more acid than this sometimes did and sometimes did not ferment without liquor potassæ; but when the acidity exceeded what would be equivalent to two per cent. of liquor potassæ, the fluid did not ferment under the influence of the high generating temperature alone. Urines of all degrees of acidity, however, were found to ferment under the combined

influence of heat and liquor potassæ added afterwards, in the manner already detailed *.

It was further ascertained that the acidity of some specimens of urine was lessened during the process of ebullition (owing to the deposition of acid phosphates); and such urines boiled for six minutes were found to ferment in a much shorter time than when they were only boiled for three minutes. The prolongation to this extent of the germ-destroying temperature actually hastened the subsequent process of fermentation.

Interpretation of Results.

The generally received belief that all *Bacteria* and their germs are killed by exposing them even for a minute or two to the temperature of 212° F. (100° C.) has of late been strongly reinforced by Professor Tyndall. The fact, therefore, of the fermentation of some specimens of boiled acid urine, with the appearance of swarms of *Bacteria*, under the influence of the high generating temperature of 122° F. (50° C.); is inexplicable except upon the supposition that fermentation has in these instances been initiated without the aid of living germs, and that the organisms first appearing in such fluids have been evolved therein.

If the author's further position (Proceedings of Royal Society, Nos. 143 and 145, 1873), that *Bacteria* and their germs are killed in fluids whether acid or alkaline at a temperature of 158° F. (70° C.), is correct, then the occurrence of fermentation in the previously neutralized boiled urine would similarly disprove the exclusive germ-theory of fermentation and establish the occurrence of Archebiosis.

Any difficulty which might have been felt by others in accepting the above interpretation of the results of these latter experiments—in face of the view held by M. Pasteur that some *Bacteria*-germs are able in neutral fluids to survive an exposure to a heat of 212° F. (100° C.)—has been fairly met and nullified by the experiments (devised for the purpose) in which the urine was boiled in the acid state and subsequently fertilized by the addition of boiled liquor potassæ.

If we look at these latter experiments from an independent point of view, it will be found that this fertilization of a previously barren fluid by boiled liquor potassæ must be explained by one or other of three hypotheses :—

1st Hypothesis. *The boiled liquor potassæ may act as a fertilizing agent because it contains living germs.*—However improbable this hypothesis may seem on the face of it, it has been actually disproved by many of the experiments recorded in this memoir. These experiments show that boiled liquor potassæ will only act as a fertilizing agent when it is added in certain proportions. If it acted as a mere germ-containing medium, a

* In the urine of highest acidity with which experiment has been made, twenty minims of liquor potassæ to the fluid ounce (about 4 per cent.) was required for neutralization.

single drop of it would suffice to infect many ounces, a gallon, or more of the sterilized fluid. This, however, is never the case; it only fertilizes the barren urine when it is added in a proportion dependent upon the precise acidity and quantity of the fluid with which experiment is being made.

2nd Hypothesis. *The fertilizing agent may act by reviving germs hitherto presumed to have been killed in the boiled acid urine.*—The acceptance of this hypothesis would involve a general recantation of the previously received conclusion that *Bacteria* and their germs are killed by boiling them in acid fluids. But such a recantation would be scarcely justifiable or acceptable unless based upon good independent evidence.

The possibility, however, of accepting this second hypothesis is still further closed by the results of experiments in which a slight excess of liquor potassæ was added to the boiled urine. Such fluids invariably remained barren. Yet it can be easily shown that the mere development and growth of *Bacteria*-germs may take place both quickly and freely in boiled urine containing a very large excess of liquor potassæ*. It would seem that this agent mixed with boiled urine in quantity slightly more than is needed for neutralization, prevents the *origination* of living matter therein, although even when in considerable excess the same agent affords no obstacle to the development, growth, and multiplication of germs purposely added thereto.

In the face of these facts it would seem impossible to accept this second hypothesis, even if it had not been independently negated by the great mass of evidence (lately reinforced by the experiments of Professor Tyndall) to the effect that *Bacteria* and their germs are really killed in fluids raised for a few minutes to the boiling-point (212° F.).

3rd Hypothesis. *The fertilizing agent acts by helping to initiate chemical changes of a fermentative character in a fluid devoid of living organisms or living germs.*—If the cause of the fermentation of the fluids in question does not exist in the form of living organisms or germs either in the fertilizing agent itself or in the medium fertilized, then it must be found in some chemical reactions set up between the boiled liquor potassæ and the boiled urine.

The experiments in which liquor potassæ is added to urine in definite proportions before and after it has been boiled with the result of inducing fermentation in the otherwise barren fluids, as well as those in which unaltered urine ferments under the influence of the high generating temperature of 122° F. (50° C.), all alike, therefore, point to the same conclusion. They show, as other experiments have done, that an exclusive germ-theory of fermentation is untenable; and they further show that

* A mixture of one part of liquor potassæ to seven of boiled urine poured into a bottle which has been washed with ordinary tap-water will, within forty-eight hours, swarm with *Bacteria* if it is kept at a temperature of 122° F.

living matter may and does originate independently during the progress of fermentation in previously germless fluids.

As a result of the fermentative changes taking place in boiled urine or other complex organic solutions, many new chemical compounds are produced: gases are given off, or these with other soluble products mix imperceptibly with the changing and quickening mother-liquid, in all parts of which certain insoluble products also make their appearance. Such insoluble products reveal themselves to us as specks of protoplasm, that is of 'living' matter; they gradually emerge into the region of the visible, and speedily assume the well-known forms of one or other variety of *Bacteria*.

These insoluble particles would thus in their own persons serve to bridge the narrow gulf between certain kinds of 'living' and of 'dead' matter, and thereby afford a long-sought for illustration of the transition from chemical to so-called 'vital' combinations.

XV. "On the Variations of the Daily Range of Atmospheric Temperature as recorded at the Kew Observatory." By BALFOUR STEWART, LL.D., F.R.S., Professor of Natural Philosophy at Owens College, Manchester. Received May 25, 1876.

(Abstract.)

The daily temperature-range was selected as an element which affords a good indication of the varying meteorological activity of the place, and the observations of which can be easily made and reduced.

The records of the Kew Observatory were chosen because there the atmospheric temperature has been very carefully observed during a long series of years. The writer desires to thank the Kew Committee for giving him access to the records of the maximum and minimum temperatures taken at the Kew Observatory.

Twenty-one years of these records have been reduced, beginning with the year 1855 and ending with 1875. Two complete sun-spot periods are embraced in these observations.

The first Table exhibits (a point already well known) the annual variation of the temperature-range, which is greatest in summer and least in winter.

The same Table shows that the yearly means of this element exhibit considerable fluctuations amongst themselves. Thus we have corresponding to the years 1856, 1866, and 1875 the values $12^{\circ}69$, $13^{\circ}61$, and $13^{\circ}25$ respectively, while corresponding to the years 1859 and 1870 we have the values $14^{\circ}52$ and $15^{\circ}63$.

Inasmuch as the three former are years of minimum, and the two latter years of maximum sun-spots, this would seem to show that the daily temperature-range is least for minimum and greatest for maximum sun-spot years.

